

136  
135

## CLAIMS

1. A method of constructing a variant of a parent *Coprinus* laccase, which variant has laccase activity and increased oxidation potential and/or altered pH optimum and/or altered mediator pathway and/or altered  $O_2/OH^-$ -pathway as compared to said parent laccase, which method comprises

i) analysing the three-dimensional structure of the parent *Coprinus* laccase to identify at least one amino acid residue or at least one structural part of the *Coprinus* laccase structure, which amino acid residue or structural part is believed to be of relevance for altering the oxidation potential and/or altering the pH optimum and/or altering the mediator pathway and/or altering the  $O_2/OH^-$ -pathway of the parent *Coprinus* laccase (as evaluated on the basis of structural or functional considerations),

ii) constructing a *Coprinus* laccase variant, which as compared to the parent *Coprinus* laccase, has been modified in the amino acid residue or structural part identified in i) so as to alter the oxidation potential and/or alter the pH optimum and/or alter the mediator pathway and/or alter the  $O_2/OH^-$ -pathway, and, optionally,

iii) testing the resulting *Coprinus* laccase variant with respect to oxidation potential and/or pH optimum and/or mediator pathway and/or  $O_2/OH^-$ -pathway.

2. A variant of a parent *Coprinus* laccase, which variant has increased oxidation potential and comprises a mutation in a position corresponding to at least one of the following positions in SEQ ID No. 1:

G411A, V, P, L, I, F, Y, W;

G412A, V, P, L, I, F, Y, W;

V409P, L, I, F, Y, W;

T257A, V, P, L, I, F, Y, W;

137  
~~136~~

10

15 180-181;

257;

352-353;

20 357-358;

409-416;

470-490.

179-182;

223;

30 281-282;

353-358;

410-412;

472;

474-475;

35 477-478.

5. A method of constructing a variant of a parent *Coprinus*-like laccase, which variant has laccase activity and increased

138  
137

oxidation potential and/or changed pH optimum and/or altered mediator pathway and/or altered  $O_2/OH^-$ -pathway as compared to said parent laccase, which method comprises

i) comparing the three-dimensional amino acid structure of the  
5 *Coprinus* laccase with an amino acid sequence of a *Coprinus*-like laccase,

ii) identifying a part of the *Coprinus*-like laccase amino acid sequence which is different from the *Coprinus* laccase amino  
10 acid sequence and which from structural or functional considerations is contemplated to be responsible for differences in the stability of the *Coprinus* and *Coprinus*-like laccase,

15 iii) modifying the part of the *Coprinus*-like laccase identified in ii) whereby a *Coprinus*-like laccase variant is obtained, which has an increased oxidation potential and/or changed pH optimum and/or altered mediator pathway and/or altered  $O_2/OH^-$ -pathway as compared to the parent *Coprinus*-like laccase, and  
20 optionally,

iv) testing the resulting *Coprinus*-like laccase variant with respect to oxidation potential and/or pH optimum and/or mediator pathway and/or  $O_2/OH^-$ -pathway.

25

6. The method according to claim 5, wherein, in step iii), the part of the *Coprinus*-like laccase is modified so as to resemble the corresponding part of the *Coprinus* laccase.

30 7. The method according to claim 5 or 6, wherein, in step iii), the modification is accomplished by deleting one or more amino acid residues of the part of the *Coprinus*-like laccase to be modified; or the modification is accomplished by replacing one or more amino acid residues of the part of the *Coprinus*-like  
35 laccase to be modified with the amino acid residues occupying corresponding positions in the *Coprinus* laccase; or the

139  
138

modification is accomplished by insertion of one or more amino acid residues present in the *Coprinus* laccase into a corresponding position in the *Coprinus*-like laccase.

5 8. The method according to any of claims 5-7, wherein the *Coprinus*-like laccase is selected from the group consisting of *Polyporus pinsitus* laccase, *Phlebia radiata* laccase, *Rhizoctonia solani* laccase, *Scytalidium thermophilum* laccase and *Myceliophthora thermophila* laccase.

10

9. The method according to claim 1 or 5, wherein the parent *Coprinus* laccase is derived from a strain of *Coprinus cinereus*.

10. The method according to claim 9, wherein the parent  
15 *Coprinus* laccase is derived from *Coprinus cinereus* IFO 8371.

11. A variant of a parent *Polyporus pinsitus* (I) laccase, which variant has an increased oxidation potential and comprises a mutation in a position corresponding to at least one of the  
20 following positions in SEQ ID No. 2:

A390V, P, L, I, F, Y, W;

G392A, V, P, L, I, F, Y, W;

E460D.

25 12. A variant of a parent *Polyporus pinsitus* (I) laccase, which variant has an altered pH optimum and comprises a mutation in a position corresponding to at least one of the following positions in SEQ ID No. 2:

E460L, I, F, M, S;

30 F463L, M.

13. A variant of a parent *Polyporus pinsitus* (I) laccase, which variant has an altered mediator efficiency and comprises a mutation in a position corresponding to at least one of the  
35 following positions in SEQ ID No. 2:

G392A;

002227 03222700

Sub  
a2

A461T,S;  
N260Q,Y;  
G165K,R.

5 14. A variant of a parent *Polyporus pinsitus* (I) laccase, which variant has an altered O<sub>2</sub>/OH<sup>-</sup> pathway and comprises a mutation in a position corresponding to at least one of the following positions in SEQ ID No. 2:

F81D,E;  
10 L112D,E;  
A80D,E.

15. A variant of a parent *Myceliophthora thermophila* laccase, which variant has an increased oxidation potential and 15 comprises a mutation in a position corresponding to at least one of the following positions in SEQ ID No. 10:

G511A,V,P,L,I,F,Y,W;  
T428A,V,P,L,I,F,Y,W;  
S510A,V,P,L,I,F,Y,W;  
20 D106A,V,P,L,I,F,Y,W;  
N109A,V,P,L,I,F,Y,W,Q;  
L500I,F,Y,W;  
A108V,P,L,I,F,Y,W;  
G514A,V,P,L,I,F,Y,W.

25

16. A variant of a parent *Myceliophthora thermophila* laccase, which variant has an altered pH optimum and comprises a mutation in a position corresponding to at least one of the following positions in SEQ ID No. 10:

30 192-193;  
234-236;  
269;  
293-294;  
364-365;  
35 372-373;  
426-433;  
503-513.

sub  
az  
cont

235;

~~365-373;~~

505;

507-508;

510-511.

[illegible]

20 N109D;

H93E;

H95E;

M433E;

M480E.

25

19. A DNA construct comprising a DNA sequence encoding a laccase variant according to claim 2 or claims 11-18.

20. A recombinant expression vector which carries a DNA con-  
30 struct according to claim 19.

21. A cell which is transformed with a DNA construct according to claim 19 or a vector according to claim 20.

35 22. A cell according to claim 21, which is a microorganism.

23. A cell according to claim 22, which is a bacterium or a fungus.

142  
141

24. A cell according to claim 23, which is an *Aspergillus niger* or an *Aspergillus oryzae* cell.

5 25. Use of a laccase variant according to claim 2 or claims 11-18 for oxidizing a substrate.

26. Use of a laccase variant according to claim 2 or claims 11-18 for dye transfer inhibition.

10

27. Use of a laccase variant according to claim 2 or claims 11-18 for bleaching textiles, in particular for bleaching denim.

28. A detergent additive comprising a laccase variant according to claim 2 or claims 11-18 in the form of a non-dusting granulate, a stabilised liquid or a protected enzyme.

29. A detergent additive according to claim 28, which additionally comprises one or more other enzyme such as a protease, a lipase, an amylase, and/or a cellulase.

30. A detergent composition comprising a laccase variant according to claim 2 or claims 11-18 and a surfactant.

25 31. A detergent composition according to claim 30 which additionally comprises one or more other enzymes such as a protease, a lipase, an amylase and/or a cellulase.

Add B1